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- (72) Inventors; and  
(75) Inventors/Applicants (*for US only*): EDENS, Luppó [NL/NL]; Hoflaan 118, NL-3062 JL Rotterdam (NL). VAN DIJK, Albertus, Alard [NL/NL]; Cederdreef 32, NL-3137 PB Vlaardingen (NL). HOPPER, Sylvia [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). ILGENFRITZ, Hilmar [DE/DE]; Lochhamer Strasse 11, 82152 Martinsried (DE). KEMMNER, Wolfram [DE/DE]; Boschetsrieder Str. 97 B, 81379 München (DE). KLUGBAUER, Sabine [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). FRITZ, Andreas [DE/DE]; Lochhamer Strasse 11, 82152 Martinsried (DE). MAIER, Dieter [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). TAN, Pamela [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). SPREAFICO, Fabio [IT/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). STOCK, Alexander [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). KIMPEL, Erik [DE/DE]; Sämannstr. 100, 81249 München (DE). HEINRICH, Oliver [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). GERHARD, Beatrix [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). EHRENREICH, Armin [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). LOSKO, Sascha [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). WAGNER, Christian [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). FOLKERS, Ulrike [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). ALBANG, Richard [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE).
- (74) Agents: MATULEWICZ, Emil, Rudolf, Antonius et al.; DSM Intellectual Property, Delft Office (600-0240), P.O. Box 1, NL-2600 MA Delft (NL).
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[Continued on next page]

(54) Title: NOVEL PECTINASES AND USES THEREOF

(57) Abstract: The invention relates to newly identified polynucleotide sequences comprising genes that encode novel pectinases isolated from *Aspergillus niger*. The invention features the full length nucleotide sequence of the novel genes, the cDNA sequences comprising the full length coding sequence of the novel pectinases as well as the amino acid sequence of the full-length functional proteins and functional equivalents thereof. The invention also relates to methods of using these enzymes in industrial processes and methods of diagnosing fungal infections. Also included in the invention are cells transformed with a polynucleotide according to the invention and cells wherein a pectinase according to the invention is genetically modified to enhance or reduce its activity and/or level of expression.



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ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,  
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Published:**

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/05726

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/40 C12N15/52 C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 447 862 A (MEYHACK BERND ET AL) 5 September 1995 (1995-09-05) see SEQ ID NO:7examples 12.3,12.4,12.5,13.2,14.1,14.2,14.3 -----	1-4, 9-11,18
Y	WO 94/14966 A (ROLIN CLAUS ;MUELLER YVONNE (NL); VISSER JACOB (NL); GIST BROCADES) 7 July 1994 (1994-07-07) examples 1.1,4.1,4.2,4.3,5.1 -----	5-8, 12-17, 19-21
Y	MILL P J: "The pectic enzymes of Aspergillus niger. A mercury-activated exopolysaccharuronase." THE BIOCHEMICAL JOURNAL. ENGLAND JUN 1966, vol. 99, no. 3, June 1966 (1966-06), pages 557-561, XP009018280 ISSN: 0264-6021 the whole document -/--	5-8, 12-17, 19-21

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

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European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Guarinos Viñals, E

## INTERNATIONAL SEARCH REPORT

International Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>-&amp; MILL P J: "The pectic enzymes of <i>Aspergillus niger</i>. A second exopolygalacturonase." THE BIOCHEMICAL JOURNAL. ENGLAND, vol. 99, no. 3, - June 1966 (1966-06) pages 562-565, XP009018279 the whole document</p> <p>-----</p> <p>OMRAN H ET AL: "SOME RESULTS OF THE ISOLATION OF DISCRETE ENZYMES EXOPOLY GALACTURONASE AND PECTINESTERASE FROM A COMMERCIAL PECTIC ENZYME PREPARATION I. ISOLATION PURIFICATION AND CHARACTERIZATION OF AN EXOPOLY GALACTURONASE EXOPG" LEBENSMITTEL-WISSENSCHAFT &amp; TECHNOLOGIE, vol. 19, no. 6, 1986, pages 457-463, XP009018347 ISSN: 0023-6438 the whole document</p> <p>-----</p> <p>HARA T ET AL: "CLARIFICATION OF APPLE JUICE WITH POLY GALACTURONASE OF ASPERGILLUS-NIGER AND PECTINESTERASE OF ASPERGILLUS-ORYZAE AND THEIR PROTOPECTIN ADSORPTION AND DEGRADATION BEHAVIOR" JOURNAL OF THE JAPANESE SOCIETY FOR FOOD SCIENCE AND TECHNOLOGY, vol. 33, no. 5, 1986, pages 336-341, XP009018288 ISSN: 0029-0394 table 1</p> <p>-----</p> <p>US 6 197 566 B1 (HALKIER TORBEN ET AL) 6 March 2001 (2001-03-06)</p> <p>-----</p>	

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Information on patent family members

International Application No

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International Application No

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 03/05726

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

s. PCT/ISA/210 annex

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-21 partially

a polynucleotide identified by SEQ NO:1 or SEQ ID NO:33,  
encoding the polypeptide identified by SEQ ID NO:65 (PEC1),  
a vector comprising said polynucleotides, a method for  
manufacturing said polynucleotides or said polypeptide, a  
recombinant host cell comprising said polynucleotide,  
antibodies reactive with said polypeptide and a fusion  
protein comprising said polypeptide.

Inventions 2-32: claims 1-21 partially

as invention 1 but related to SEQ ID NOs: 2-32, SEQ ID NOs:  
34-64 and SEQ ID NOs: 66-96, wherein  
invention 2 relates to SEQ ID NOs: 2, 34 and 66 (PEC2)  
invention 3 relates to SEQ ID NOs: 3, 35 and 67 (PEC3)  
.....  
invention 32 relates to SEQ ID NOs: 32, 64 and 96 (PEC 32).